

Figure 1 BLOSUM62 Substitution Matrix

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	9	- 1	- 1	- 3	0	- 3	- 3	- 3	- 4	- 3	- 3	- 3	- 3	- 1	- 1	- 1	- 1	- 2	- 2	- 2
S	- 1	4	1	- 1	1	0	1	0	0	0	- 1	- 1	0	- 1	- 2	- 2	- 2	- 2	- 2	- 3
T	- 1	1	4	1	- 1	1	0	1	0	0	0	- 1	0	- 1	- 2	- 2	- 2	- 2	- 2	- 3
P	- 3	- 1	1	7	- 1	- 2	- 1	- 1	- 1	- 1	- 2	- 2	- 1	- 2	- 3	- 3	- 2	- 4	- 3	- 4
A	0	1	- 1	- 1	4	0	- 1	- 2	- 1	- 1	- 2	- 1	- 1	- 1	- 1	- 1	- 2	- 2	- 2	- 3
G	- 3	0	1	- 2	0	6	- 2	- 1	- 2	- 2	- 2	- 2	- 2	- 3	- 4	- 4	0	- 3	- 3	- 2
N	- 3	1	0	- 2	- 2	0	6	1	0	0	- 1	0	0	- 2	- 3	- 3	- 3	- 3	- 2	- 4
D	- 3	0	1	- 1	- 2	- 1	1	6	2	0	- 1	- 2	- 1	- 3	- 3	- 4	- 3	- 3	- 3	- 4
E	- 4	0	0	- 1	- 1	- 2	0	2	5	2	0	0	1	- 2	- 3	- 3	- 3	- 3	- 2	- 3
Q	- 3	0	0	- 1	- 1	- 2	0	0	2	5	0	1	1	0	- 3	- 2	- 2	- 3	- 1	- 2
H	- 3	- 1	0	- 2	- 2	- 2	1	1	0	0	8	0	- 1	- 2	- 3	- 3	- 2	- 1	2	- 2
R	- 3	- 1	- 1	- 2	- 1	- 2	0	- 2	0	1	0	5	2	- 1	- 3	- 2	- 3	- 3	- 2	- 3
K	- 3	0	0	- 1	- 1	- 2	0	- 1	1	1	- 1	2	5	- 1	- 3	- 2	- 3	- 3	- 2	- 3
M	- 1	- 1	- 1	- 2	- 1	- 3	- 2	- 3	- 2	0	- 2	- 1	- 1	5	1	2	- 2	0	- 1	- 1
I	- 1	- 2	- 2	- 3	- 1	- 4	- 3	- 3	- 3	- 3	- 3	- 3	- 3	1	4	2	1	0	- 1	- 3
L	- 1	- 2	- 2	- 3	- 1	- 4	- 3	- 4	- 3	- 2	- 3	- 2	- 2	2	2	4	3	0	- 1	- 2
V	- 1	- 2	- 2	- 2	0	- 3	- 3	- 3	- 2	- 2	- 3	- 3	- 2	1	3	1	4	- 1	- 1	- 3
F	- 2	- 2	- 2	- 4	- 2	- 3	- 3	- 3	- 3	- 3	- 1	- 3	- 3	0	0	0	- 1	6	3	1
Y	- 2	- 2	- 2	- 3	- 2	- 3	- 2	- 3	- 2	- 1	2	- 2	- 2	- 1	- 1	- 1	- 1	3	7	2
W	- 2	- 3	- 3	- 4	- 3	- 2	- 4	- 4	- 3	- 2	- 2	- 3	- 3	- 1	- 3	- 2	- 3	1	2	1 1

Figure 2. Blast alignment of Pfu (query 1) against Deep Vent (subject 1)

>gi|2494186|sp|Q51334|DPOL\_PYRSD DNA polymerase (Deep Vent DNA polymerase)  
[Contains: Endonuclease  
PI-PspI (Psp-GDB pol intein)]  
Length = 1312

Score = 816 bits (2109), Expect = 0.0  
Identities = 414/493 (83%), Positives = 459/493 (92%)

Query: 1 MILDVDYITEEGKPVIRLFKKENGKFKIEHRTFRPYIYALLRDDSKIEEVKKITGERHG 60  
MILD DYITE+GKP+IR+FKKENG+FK+E+DR FRPYIYALL+DDS+I+EV+KIT ERHG  
Sbjct: 1 MILDADYITEDGKPIIRIFKKENGEPKVEYDRNFRPYIYALLKDDSQIDEVRKITAERHG 60

Query: 61 KIVRIVDVEKVEKKFLGKPITVWKLYLEHPQDVPTIREKVRHAPAVDIFEYDIPFAKRY 120  
KIVRI+D EKV KKFLG+PI VW+LY EHPQDVP IR+K+REH AV+DIFEYDIPFAKRY  
Sbjct: 61 KIVRIIDAOKVRKKFLGRPIEVWRLYFEHPQDVPAIRDKIREHSAVIDIFEYDIPFAKRY 120

Query: 121 LIDKGLIPMEGEEELKILAFDIETLYHEGEEFEGKGPIMISYADENEAKVITWKNIDLPY 180  
LIDKGLIPMEG+EELK+LAFDIETLYHEGEEF KGPIMISYADE EAKVITWK IDLPY  
Sbjct: 121 LIDKGLIPMEGDEELKLLAFDIETLYHEGEEFAKGPIMISYADEEEAKVITWKKIDLPY 180

Query: 181 VEVVSSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLTIGRDGSEPK 240  
VEVVSSSEREMIKRFL++IREKDPD+I+TYNGDSFD PYL KRAEKLGIKL +GRDGSEPK  
Sbjct: 181 VEVVSSSEREMIKRFLKVIREKDPDVIITYNGDSFDLPYLVKRAEKLGIKLPLGRDGSEPK 240

Query: 241 MQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKKEKVYADEIAKAW 300  
MQR+GDMTAVE+KGRIHFDLYHVI RTINLPTYTLEAVYEAIFGKPKKEKVYA EIA+AW  
Sbjct: 241 MQRIGDMTAVEIKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKKEKVYAEIAEAW 300

Query: 301 SGENLERVAKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSSTGNLVEWFLLRK 360  
+G+ LERVAKYSMEDAK TYELG+EF PME QLSRLVGQPLWDVSRSSSTGNLVEW+LLRK  
Sbjct: 301 TGKGLERVAKYSMEDAKVTYELGREFFPMEAQLSRLVGQPLWDVSRSSSTGNLVEWYLLRK 360

Query: 361 AYERNEVAPNKPSEEEYQRRRESYTGGFVKEPEKGLWENIVYLDLFRALYPSIIITHNVS 420  
AYERNE+APNKP E EY+RRRESY GG+VKEPEKGLWE +V LDFR+LYPSIIITHNVS  
Sbjct: 361 AYERNELAPNKPDEREYERRRESYAGGYVKEPEKGLWEGLVSLDFRSLYPSIIITHNVS 420

Query: 421 PDTLNLEGCKKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQKIKTKMKETQDPIEKILL 480  
PDTLN EGC+ YD+AP+VGHKFCKD PGFIPSLL LL+ERQ+IK KMK ++DPIEK +L  
Sbjct: 421 PDTLNREGCREYDVAPEVGHKFCKDFPGFIPSLKRLLLDERQEIKRKMKASKDPIEKML 480

Query: 481 DYRQKAIKLLANS 493  
DYRQ+AIK+LANS  
Sbjct: 481 DYRQRAIKILANS 493

Figure 2--continued. Blast alignment of Pfu (query 1) against Deep Vent (subject 1)

Score = 473 bits (1216), Expect = e-133  
Identities = 248/283 (87%), Positives = 269/283 (94%)

Query: 492 NSFYGYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLYIDTDGLYATIPG 551  
NS+YGYGYAKARWYCKECAESVTAWGR+YIE V KELEEKFGFKVLYIDTDGLYATIPG  
Sbjct: 1029 NSYGYGYGYAKARWYCKECAESVTAWGREYIEFVRKELEEKFGFKVLYIDTDGLYATIPG 1088

Query: 552 GESEEEKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEI 611  
+ EEIKKKALEFV YIN+KLPGLLELEYEGFY RGFFVTKK+YA+IDEEGK+ITRGLEI  
Sbjct: 1089 AKPEEEKKKALEFVDYINAKLPGLLELEYEGFYVRGFFVTKKKYALIDEEGKIITRGLEI 1148

Query: 612 VRRDWSEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPL 671  
VRRDWSEIAKETQA+VLE ILKHG+VEEAV+IVKEV +KL+ YEIPPEKL IYEQITRPL  
Sbjct: 1149 VRRDWSEIAKETQAKVLEAILKHGNVEEAVKIVKEVTEKLSKYEIPPEKLVIYEQITRPL 1208

Title: METHODS OF MAKING HYBRID PROTEINS  
Inventor: Peter B. Vander Horn. - Filed: Herewith  
Attorney Docket No.: 020130-001420US - Sheet 3 of 20

Query: 672 HEYKAIGPHVAVAKLAAGVKIKPGMVIGYIVLRGDGPISNRAILAEEDPKKHKYDAE 731  
HEYKAIGPHVAVAK+LAA+GVK++PGMVIGYIVLRGDGPIS RAILAEE+D +KKHYDAE  
Sbjct: 1209 HEYKAIGPHVAVAKRLAARGVKVRPGMVIGYIVLRGDGPISKRAILAEEDLRKKHYDAE 1268

Query: 732 YYIENQVLPVAVLRILEGFGYRKEDLRYQKTRQVGLTSLWLNKK 774  
YYIENQVLPVAVLRILE FGYRKEDLR+QKT+Q GLT+WLNKK  
Sbjct: 1269 YYIENQVLPVAVLRILEAFGYRKEDLRWQKTQTGLTAWLNKK 1311

Figure 3. Assembly of the oligonucleotides into library fragments.

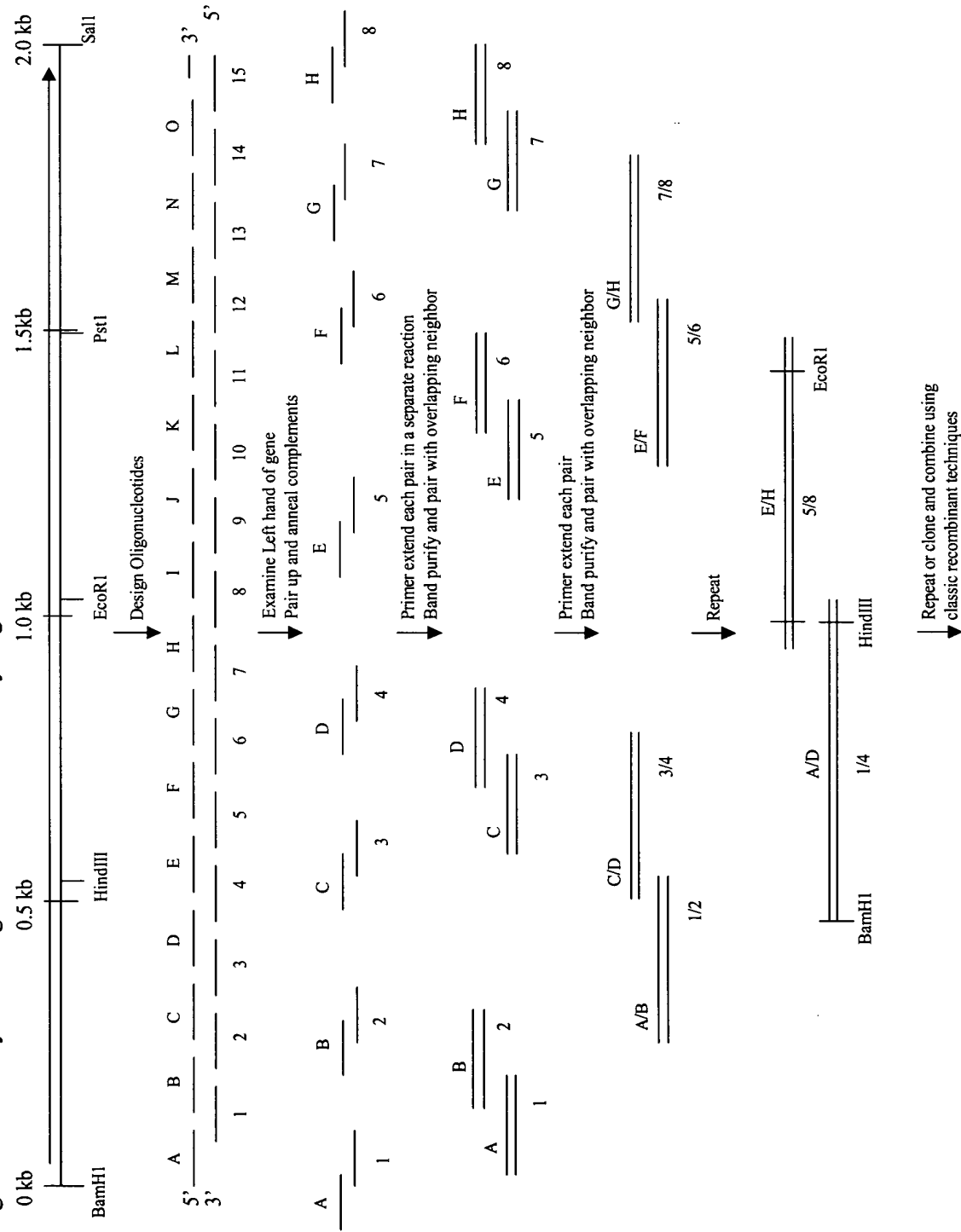


FIG. 4

E coli dut polypeptide sequence:

MKKIDVKILDPRVGKEFPLPTYATSGSAGLDLRACLND AVELAPGDTTLVPTGLAIHIADPSLAAMMLPRSGLGHKHG  
IVLGNLVGLIDSDYQGQLMISVWNRGQDSFTIQPGERIAQMIFVPVVQAEFNLVEDFDATDRGEGGFGHSGRQ

AAD polypeptide sequence:

MSKVILKIKRLPHAQDLPLPSYATPHSSGLDLRAAIEKPLKIKPFERVL IPTGLILEIPEGYEQVRPRSGLAWKKGL  
TVLNAPGTIDADYRGEVKVILVNLGNEEVVIERGERIAQLVIAPVQRVEVVEVEEVSQTQRGEGGFGSTGTK

Alignment:

Identities = 61/149 (40%), Positives = 91/149 (60%), Gaps = 1/149 (0%)

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Query: 1  MKKIDVKILDPRVGKEFPLPTYATSGSAGLDLRACLND AVELAPGDTTLVPTGLAIHIAD 60
          M K+ +KI      ++ PLP+YAT  S+GLDLRA +   +++ P +  L+PTGL + I +
Sbjct: 1  MSKVILKIKRLPHAQDLPLPSYATPHSSGLDLRAAIEKPLKIKPFERVL IPTGLILEIPE 60

Query: 61  PSLAAMMLPRSGLGHKHGIVLGNLVGLIDSDYQGQLMISVWNRGQDSFTIQPGERIAQMI 120
          + PRSGL  K G+ + N  G ID+DY+G++ + + N G +   I+ GERIAQ++
Sbjct: 61  -GYEQVVRPRSGLAWKKGLTVLNAPGTIDADYRGEVKVILVNLGNEEVVIERGERIAQLV 119

Query: 121 FVPVVQAEFNLVEDFDATDRGEGGFGHSG 149
          PV + E   VE+   T RGEGGFG +G
Sbjct: 120 IAPVQRVEVVEVEEVSQTQRGEGGFGSTG 148
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**FIGURE 5**  
**5A. Aligned parental sequence showing all possible codons**

ECD	1	M	K	K	I	D	V	K	I	L	D	P	R	V	G	K	E	F	P	L	P	T	Y	A	T	S	G	S	A	G	L
		ATG	AAA	AAA	AAA	ATT	GAT	GTG	AAA	ATT	CTG	GAT	CCG	CGT	GTG	GGC	AAA	GAA	TTT	CCG	CTG	CCG	ACC	TAT	GCG	ACC	AGC	GGC	AGC	GCG	CTG
		AAG	AAG	ATC	GAC	GTT	AAG	ATC	TTA	GAC	CCT	CGC	GTT	GGT	GGT	AAG	GAG	TTC	CCT	TTA	CCT	ACG	TAC	GCC	ACG	TCT	GGT	TCT	GCC	GGT	TTA
				ATA		ATA	TTG				CCA	CGG	GTC	GGG					CCA	TTG	CCA	ACT	GCA	ACT	AGT	GGG	AGT	GCA	GGG	TTG	
								GTA			CCC	CGA	GTA	GGA					CCC	CTT	CCC	ACA	GCT	ACA	TCA	GGA	TCA	GCT	GGA	CTT	
										CTC		AGA							CTC						TCC		TCC			CTC	
										CTA		AGG							CTA						TCG		TCG			CTA	

AAD	1	M	S	K	V	I	L	K	I	K	R	L	P	H	A	Q	D	L	P	L	P	S	Y	A	T	P	H	S	S	G	L
ATG	AGC	AAA	GTG	ATT	CTG	AAA	ATT	AAA	CGT	CTG	CCG	CAT	GCG	CAG	GAT	CTG	CCG	CTG	CCG	AGC	AGC	TAT	GCG	ACC	CCG	CAT	AGC	AGC	GGC	CTG	
	TCT	AAG	GTT	ATC	TTA	AAG	ATC	AAG	CGC	TTA	CCT	CAC	GCC	CAA	GAC	TTA	CCT	TTA	CCT	TCT	TAC	GCC	ACG	CCT	CAC	TCT	TCT	GGT	TTA		
	AGT	GTC	ATA	TTG		ATA			CGG	TTG	CCA		GCA			TTG	CCA	TTG	CCA	AGT		GCA	ACT	CCA	AGT	AGT	GGG	TTG			
	TCA	GTA		CTT					CGA	CTT	CCC		GCT			CTT	CCC	CTT	CCC	TCA		GCT	ACA	CCC	TCA	TCA	GGA	CTT			
	TCC			CTC					AGA	CTC						CTC		CTC		TCC					TCC	TCC		CTC			
	TCG			CTA					AGG	CTA						CTA		CTA		TCG					TCG	TCG		CTA			

### 5B. The minimal encoding sequence

	Min.	E.	Seq.	ATG	AAA	AAA	GAT	GTG	AAA	ATT	CTG	GAT	CCG	CGT	GTT	GGC	AAA	GAA	TTT	CCG	CTG	CCG	ACC	TAT	GCG	ACC	TCT	GGC	AGC	GCA	GGC	CTG
				GC			G	AT	C			AA	CG	T	C	CA	C	C	T	A			G				C	CA		T		
M	K	K	I	D	V	K	I	L	D	P	R	V	G	K	E	F	P	L	P	L	P	T	Y	A	T	S	G	S	A	G	L	
	S		V	I	L			K	R	L	P	H	A	Q	D	L						S			P	H		S				
R			V					Q	G			D														D						
N			N					M	H			L														R						

5C. A minimal encoding sequence after the removal of non-similar degeneracies; selection of ADD parent residues at particular sites

[illegible]

AAD	31	D	L	R	A	A	I	E	K	P	L	K	I	K	P	F	E	R	V	L	I	G	L	E	I	P	E	G
		GAT	CTG	CGT	CGG	CGC	ATT	GAA	AAA	CCG	CTG	AAA	ATT	AAA	CCG	TTT	GAA	CGT	GTG	CTG	ATT	GGC	CTG	GAA	ATT	CCG	GAA	GGC
		GAC	TTA	CGC	GCC	GCC	ATC	GAG	AAG	CCT	TTA	AAG	ATC	AAG	CCT	TTC	GAG	CGC	GTT	TTA	ATC	TTA	GAG	ATC	CCT	GAG	GGT	
			TTG	CGG	GCA	GCA	ATA			CCA	TTG		ATA		CCC			CGG	GTC	TTG	ATA	GGG	TTG	ATA	CCA		GGG	
		CTT	CGA	GCT	GCT					CCC	CCC							CGA	GTA	CTT		CTT			CCC		GGG	
			CTC	AGA							CTC						AGA		CTC	CTC		CTC					GGG	
		CTA	AGG								CTA						AGG		CTA	CTA		CTA					GGG	

**5B--continued**

**5C--continued**

[illegible]

[illegible]

**5B--continued**

**5C--continued**

TCT	---	GCG	GCG	CAG	ATG	CTG	COG	CGT	AGC	GGC	CTG	GGC	C	TGG	AAA	CAT	GGC	ATA	GTG	CTG	CTG	AAC	GCG	GTG	GGC	CTG	ATT	GAT	AGC	GAT	TAT
A		A	G	A	G	G	G	G	A	G	T	AC	G																		
S	-	A	A	Q	M	L	P	R	S	G	L	G	A	W	K	H	G	I	V	L	L	N	A	V	G	L	I	D	S	D	Y
Y		E	G		V	R										Q	K	L	T	V				P		T		A			
																N		A	A				A	L		P	M	T	G		



**5A--continued**

[illegible]

**5B--continued**

[illegible]

5C--continued

CAG	GGC	CAA	CTG	ATG	ATT	AGC	GTG	GTG	AAC	CCG	GGC	CAG	GAT	GAA	TTT	ACG	ATT	CAG	CCG	GGC	GAA	CGT	ATT	GCG	CAG	ATG	ATT	TTT	GTG	CCG
G	G	G	G	G	A	G	T	C	T	T	T	A	C	A	G	G	T	G	G	G	G	G	C	G	C	G	A	T	T	C
Q	G	Q	L	M	I	S	V	N	N	R	G	Q	D	E	F	T	I	Q	P	G	E	R	I	A	Q	M	I	F	V	P
R	E	V	K	V	I	L	L	N	E	L	N	E	H	K	V	M	A	E	R						L	V	I	A		

**5B--continued**

**5C--continued**

[illegible]

FIGURE 6

**KpnI HindIII NdeI**

1 TT GGTACC AAGCTT CAT ATG A(A/G) (A/C) AAA (A/G)TT (G/A) (A/T)T (G/C)TG AAA 38  
AA CCATGG TTCGAA GTA TAC T(T/C) (T/G) TTT (T/C)AA (C/T) (T/A)A (C/G)AC TTT  
M K/S/R/N K I/V D/I/V/N V/L K

39 ATT (C/A) (T/A)G CGT C(C/T)G C(G/C)T CAT G(G/C)C (A/C)AA GA(A/T) TT(T/A) 69  
TAA (G/T) (A/T)C GCA G(G/A)C G(C/G)A GTA C(C/G)G (T/G)TT CT(T/A) AA(A/T)  
I L/K/Q/M R P/L R/P H G/A K/Q E/D F/L

**StuI**

70 CCG CTG CCG A(C/G)C TAT GCG ACC (T/C)CT CAC AGC (G/T)CA GGC CTG GAT CTG 113  
GGC GAC GGC T(G/C)G ATA CGC TGG (A/G)GA GTG TCG (C/A)GT CCG GAC CTA GAC  
P L P T/S Y A T S/P H S A/S G L D L

114 CGT GCG (T/G) (G/C)C (C/A)TT (A/G)A(C/G) (A/G)A(T/A) (G/C)CG (G/C)TG (G/A)AA 140  
GCA CGC (A/C) (C/G)G (G/T)AA (T/C)T(G/C) (T/G)T(A/T) (C/G)GC (C/G)AC (C/T)TT  
R A C/A/S/G L/I N/E/K/D D/K/N/E A/P V/L E/K

141 (C/A)TT (G/A) (C/A)G CCG (G/T) (G/T)T GA(T/A) A(C/G)G (A/G) (C/T)G CTG 164  
(G/T)AA (C/T) (G/T)C GGC (C/A) (C/A)A CT(A/T) T(G/C)C (T/C) (G/A)C GAC  
L/I A/K/E/T P G/F/V/C D/E T/R T/V/M/A L

**AgeI**

165 (G/A)TT CCG ACC GGT CTG ATC (A/C)TT (C/G)A(T/A) ATT (G/C)CG GA(T/A) GGT 200  
(C/T)AA GGC TGG CCA GAC TAG (T/G)AA (G/C)T(A/T) TAA (C/G)GC CT(A/T) CCA  
V/I P T G L I I/L H/E/D/Q I A/P D/E G

201 T(C/A)T G(C/A)G G(C/G)G CAG (A/G)TG C(T/G)G CCG CGT AGC GGC CTG G(G/C)C 236  
A(G/T)A C(G/T)C C(G/C)C GTC (T/C)AC G(A/C)C GGC GCA TCG CCG GAC C(C/G)G  
S/Y A/E A/G Q M/V L/R P R S G L G/A

237 TGG AAA (C/A)A(T/G) GGC (A/T)TA (G/A) (T/C)G (C/G)TG CTG AAC GCG 266  
ACC TTT (G/T)T(A/C) CCG (T/A)AT (C/T) (A/G)C (G/C)AC GAC TTG CGC  
W K H/K/Q/N G I/L V/T/A/M L/V L N A

**ClaI**

267 (G/C) (T/C)G GGC (C/A) (T/C)G ATC GAT (A/G) (G/C)C GAT TAT C(A/G)G GGC 296  
(C/G) (A/G)C CCG (G/T) (A/G)C TAG CTA (T/C) (C/G)G CTA ATA G(T/C)C CCG  
V/P/A/L G L/T/P/M I D S/A/T/G D Y Q/R G

297 (C/G)AA (C/G)TG A(T/A)G (A/G)TT A(G/T)C (G/C)TG GTG AAC C(G/T)G GGC 326  
(G/C)TT (G/C)AC T(A/T)C (T/C)AA T(C/A)G (C/G)AC CAC TTG G(C/A)C CCG  
Q/E L/V M/K I/V S/I V/L N N R/L G

327 (C/A)A(G/C) GA(T/A) GAA (T/G)TT (A/G) (C/T)G ATT (C/G)AG C(C/G)G GGC GAA 356  
(G/T)T(C/G) CT(A/T) CTT (A/C)AA (T/C) (G/A)C TAA (G/C)TC G(G/C)C CCG CTT  
Q/N/H/K D/E E F/V T/V/M/A I Q/E P/R G E

**FspI**

357 CGT ATT GCG CAG (A/C)TG (A/G)TT (T/A)TT G(T/C)G CCG GTG (G/C) (T/A)G 389  
GCA TAA CGC GTC (T/G)AC (T/C)AA (A/T)AA C(A/G)C GGC CAC (C/G) (A/T)C  
R I A Q M/L I/V F/I V/A P V V/Q/E/L

390 C(A/G)G G(C/T)G GAA (T/G)TT (A/G) (A/T)T (C/G) (T/A)G GTG GAA GA(T/A) 416  
G(T/C)C C(G/A)C CTT (A/C)AA (T/C) (T/A)A (G/C) (A/T)C CAC CTT CT(A/T)  
Q/R A/V E F/V N/V/I/D L/E/Q/V V E D/E

417 (T/G)TT TCT CAG ACC (G/C)A(T/G) CGT GGC GAA GGC GGC TTT GGC TCT A(G/C)C 458  
(A/C)AA AGA GTC TGG (C/G)T(A/C) GCA CCG CTT CCG CCG AAA CCG AGA T(C/G)G  
F/V S Q T D/Q/E/H R G E G G F G S S/T

**BamHI EcoRI**

459 GGC A(G/C)A (C/A)AG TAA TGA GGATCC GAATTC TT 487  
CCG T(C/G)T (G/T)TC ATT ACT CCTAGG CTTAAG AA  
G R/T Q/K \* \*

Figure 7

10 20 30 40 50 60 70  
TTGGTACCAAGCTTCATATGARMAAARTTRWTSTGAAAATTMWGCGTCYGCSTCATGSCMAAGAWTTWCC  
AACCATGGTTCTGAAGTATACTYKTTTTYAAWASACTTTTAAKWCGCAGRCGSAGTACSGKTTCTWAAWGG

80 90 100 110 120 130 140  
GCTGCCGASCTATGCGACCYCTCACAGCKCAGGCCTGGATCTGCGTGCGKSCMTTRASRAWSCGSTGRAA  
CGACGGCTSGATACGCTGGRGAGTGTCGMGTCCGGACCTAGACGCACGCMMSGKAAYTSYTWSGCSACYTT

150 160 170 180 190 200 210  
MTTRMGCCGKKTGAWASGRYGCTGRTTCCGACCGGTCTGATCMTTSAWATTSCGGAWGGTTMTGMGGSGC  
KAAYKCGGCMMACTWTSCYRCGACYAAGGCTGGCCAGACTAGKAASTWTAASGCCTWCCAAKACKCCSCG

220 230 240 250 260 270 280  
AGRTGCKGCCGCTAGCGGCCTGGSCGTGAAAMAKGGCWTARYGSTGCTGAACGCGSYGGGCMYGATCGA  
TCYACGMCGGCGCATCGCCGGACCSGACCTTTKTMCCGWATYRCSACGACTTGCGCSRCCCGKRCTAGCT

290 300 310 320 330 340 350  
TRSCGATTATCRGGGCSAASTGAWGRTTAKCSTGGTGAACCKGGGCMASGAWGAAKTTRYGATTSAAGCSG  
AYSGCTAATAGYCCCGSTTSACTWCYAATMGSACCACTTGMCCCGKTSCTWCTTMAAYRCTAASTCGSC

360 370 380 390 400 410 420  
GGCGAACGTATTGCGCAGMTGRTTWTGCGCCGGTGSWGCRCGGYGGAAKTTRWTSWGGTGGAAGAWKTTT  
CCGCTTGCTATAACGCGTCKACYAAWAACRCGGCCACSWCGYCCRCCTTMAAYWASWCCACCTTCTWMAAA

430 440 450 460 470 480  
CTCAGACCSAKCGTGGCGAAGGCGGCTTTGGCTCTASCGGCASAMAGTAATGAGGATCCGAATTCTT  
GAGTCTGGSTMGCACCGCTTCCGCCGAAACCGAGATSGCCGTSTKTCATTACTCCTAGGCTTAAGAA

Figure 8.

14

atg atc ctg gat g(t/c)t gac tac atc act gaa ga(a/c) ggc aaa ccg (g/a)tt atc cgt (c/a)t(g/c) ttc  
M I L D V/A D Y I T E E/D G K P V/I I R L/I/M F

aaa aaa gag aac ggc (a/g)aa ttt aag (a/g)tt gag (c/t)at gat cgc a(a/c)c ttt cgt cca tac att tac gct  
K K E N G K/E F K I/V E H/Y D R N/T F R P Y I Y A

ctg ctg a(g/a)a gat gat tct (a/c)ag att ga(g/t) gaa gtt a(g/a)a aaa atc act g(g/c)t gag cgc cat ggc aag att  
L L R/K D D S K/Q I E/D E V K/R K I T G/A E R H G  
K I

214 215

gtt cgt atc (a/g)tt gat g(t/c)g gaa aag gta (g/a)(g/a)g aag aaa ttt ctg ggc a(a/g)a cca atc (a/g)(a/c)g  
V R I I/V D V/A E K V G/E/R/K K K F L G K/R P I  
K/T/E/A

gtg tgg a(g/a)a ctg tat (c/t)tc gaa cat cca caa gat gtt ccg a(t/c)t att cgc ga(g/t) aaa (g/a)tt cgc  
V W K/R L Y L/F E H P Q D V P T/A I R E/D K V/I R

gaa cat (c/t)ct gca gtt (g/a)tt gac atc ttc gaa tac gat att cca ttt gca aag cgt tac ctc atc gac aaa  
E H P/S A V V/I D I F E Y D I P F A K R Y L I D K

ggc ctg ata cca atg gag ggc ga(g/t) gaa gaa ctc aag (a/c)tc ctg gcg ttc gat ata gaa acc ctc tat  
G L I P M E G E/D E E L K I/L L A F D I E T L Y

cac gaa ggc gaa gag ttt g(g/c)t aaa ggc cca att ata atg att agc tat gca gat gaa (a/g)a(a/c) gaa gca aag  
H E G E E F G/A K G P I I M I S Y A D E K/N/E/D E A  
K

gtg att act tgg aaa aa(a/c) ata gat ctc cca tac gtt gag gtt gta tct tcc gag cgc gag atg att aag cgc  
V I T W K K/N I D L P Y V E V V S S E R E M I K R

ttt ctc a(g/a)a (g/a)tt atc cgc gag aag gat ccg gac (g/a)tt atc (g/a)tt act tat aac ggc gac tct ttt  
F L R/k V/I I R E K D P D V/I I V/I T Y N G D S F

gac (c/t)tc cca tat ctg g(t/c)g aaa cgc gca gaa aaa ctc ggt att aaa ctg (a/c)ct (a/c)tc ggc cgt gat ggt  
D F P Y L V/A K R A E K L G I K L T/P I/L G R D  
G

tcc gag ccg aag atg cag cgt (a/c)tc ggc gat atg acc gct gta gaa (g/a)tt aag ggt cgt atc cat ttc gac  
S E P K M Q R I/L G D M T A V E V/I K G R I H F D

ctg tat cat gta att (a/c)(c/g)c cgt act att aac ctc ccg act tac act ctc gag gct gta tat gaa gca att  
L Y H V I T/S/P/R R T I N L P T Y T L E A V Y E A I

ttt ggt aag ccg aag gag aag gta tac gcc (g/c)at gag att gca (a/g)ag gcg tgg gaa (a/t)cc ggt (a/g)ag  
F G K P K E K V Y A D/H E I A K/E A W E T/S G K/E

(a/g)(a/g)c ctc gag cgt gtt gca aaa tac tcc atg gaa gat gca aag g(t/c)g act tat gaa ctc ggc a(g/a)a gaa ttc  
N/G/D/S L E R V A K Y S M E D A K V/A T Y E L G R/K  
E F

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(c/t)tc cca atg gaa (a/g)(t/c)t cag ctc tct cgc ctg gtt ggc caa cca ctg tgg gat gtt tct cgt tct tcc  
L/F P M E I/T/V/A Q L S R L V G Q P L W D V S R S S

acc ggt aac ctc gta gag tgg t(t/a)t ctc ctg cgc aaa gcg tac gaa cgc aac gaa (g/c)tg gct ccg aac aag  
T G N L V E W F/Y L L R K A Y E R N E V/L A P N K

cca (t/g)(c/a)c gaa (c/g)(g/a)a gag tat (c/g)aa cgc cgt ctc cgc gag tct tac (a/g)ct ggt ggc t(t/a)t  
P S/Y/A/D E R/Q/G/E E Y Q/E R R L R E S Y T/A G G F/Y

ggt aaa gag cca gaa aag ggc ctc tgg gaa (a/g)(a/g)c (a/c)tc gtg t(c/a)c ctc gat ttt cgc (g/t)ct ctg  
V K E P E K G L W E N/S/D/G I/L V Y/S L D F R A/S L

tat ccg tct att atc att acc cac aac gtg tct ccg gat act ctc aac c(t/g)c gag ggc tgc a(g/a)a  
Y P S I I I T H N V S P D T L N L/R E G C K/R

(a/g)a(a/c) tat gat (g/a)tt gct ccg (c/g)aa gta ggc cac aag ttc tgc aag gac (a/t)tc ccg ggc ttt att  
K/N/E/D Y D V/I A P Q/E V G H K F C K D I/F P G F I

ccg tct ctc ctg (a/g)(a/g)g c(a/g)t ctg ctc ga(g/t) gaa cgc caa (a/g)ag att aag (a/c)(g/c)c aaa atg aag  
P S L L K/R/E/G R/H L L E/D E R Q K/E I K T/S/P/R K M K

g(a/c)g (a/t)cc (c/a)ag gat ccg att gaa aaa a(t/a)a (a/c)tg ctc gac tat cgc caa a(g/a)a gcg att aaa  
E/A T/S Q/K D P I E K I/K M/L L D Y R Q R/K A I K

(a/c)tc ctc gca aac tct t(a/t)t tac ggc tat tat ggc tat gca aaa gca cgc tgg tac tgt aag gag tgt gct  
L/I L A N S F/Y Y G Y Y G Y A K A R W Y C K E C A

gag tcc gtt act gct tgg ggt cgc (a/g)aa tac atc gag (c/t)tc gtg (t/c)gg aag gag ctc gaa gaa aag ttt ggc  
E S V T A W G R K/E Y I E L/F V W/R K E L E E K F G

ttt aaa gtt ctc tac att gac act gat ggt ctc tat gcg act att ccg ggt g(g/c)t (a/g)ag (c/t)ct gag  
F K V L Y I D T D G L Y A T I P G G/A E/K S/P E

1696

gaa att aag aaa aag gct ctc gaa ttt gtg aaa tac att aac (g/t)cg aag ctc ccg ggt ctc ctg gag ctc gaa  
E I K K K A L E F V K(D) Y I N A/S K L P G L L E L E

tat gaa ggc ttt tat (g/a)(t/a)g cgc ggc ttc ttc gtt acc aag aag a(g/a)a tat gcg (g/c)tg att gat gaa gaa  
Y E G F Y V/E/M/K R G F F V T K K R/K Y A V/L I D E E

ggc aaa (g/a)tt att act cgt ggt ctc gag att gtg cgc cgt gat tgg agc gaa att gcg aaa gaa act caa gct  
G K V/I I T R G L E I V R R D W S E I A K E T Q A

a(g/a)a gtt ctc gag (a/g)ct att ctc aaa cac ggc (g/a)ac gtt gaa gaa gct gtg a(g/a)a att gta aaa gaa gta  
R/K V L E T/A I L K H G D/N V E E A V R/K I V K E V

a(t/c)c (c/g)aa aag ctc (g/t)ct aa(a/c) tat gaa att ccg cca gag aag ctc g(t/c)g att tat gag cag att  
I/T Q/E K L A/S K/N Y E I P P E K L V/A I Y E Q I

act cgc ccg ctg cat gag tat aag gcg att ggt ccg cac gtg gct gtt gca aag a(g/a)a ctg gct gct a(g/a)a ggc gtg  
T R P L H E Y K A I G P H V A V A K R/K L A A K/R G V

aaa (g/a)tt a(g/a)a ccg ggt atg gta att ggc tac att gta ctc cgc ggc gat ggt ccg att agc aa(a/c) cgt gca  
K V/I R/K P G M V I G Y I V L R G D G P I S K/N R A

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att cta gct gag gaa t(t/a)c gat c(c/t)g a(g/a)a aag cac aag tat gac gca gaa tat tac att gag aac cag gtg  
I L A E E F/Y D P/L K/R K H K Y D A E Y Y I E N Q V  
2272 2273  
ctc ccg gcg gta ctc cgt att ctg gag g(g/c)t ttt ggc tac cgt aag gaa gac ctc cgc t(a/g)(c/g) caa aag  
L P A V L R I L E G/A F G Y R K E D L R Y/W/\*C Q K  
act a(g/a)a cag (g/a)(t/c)t ggc ctc act (g/t)ct tgg ctc aac att aaa aaa tcc ggt acc cac tag tgc tag cat gac  
T K/R Q V/A/I/T G L T A/S W L N I K K S G T H \*

Figure 9. A comparison of the polymerase to 3' to 5' exonuclease activity

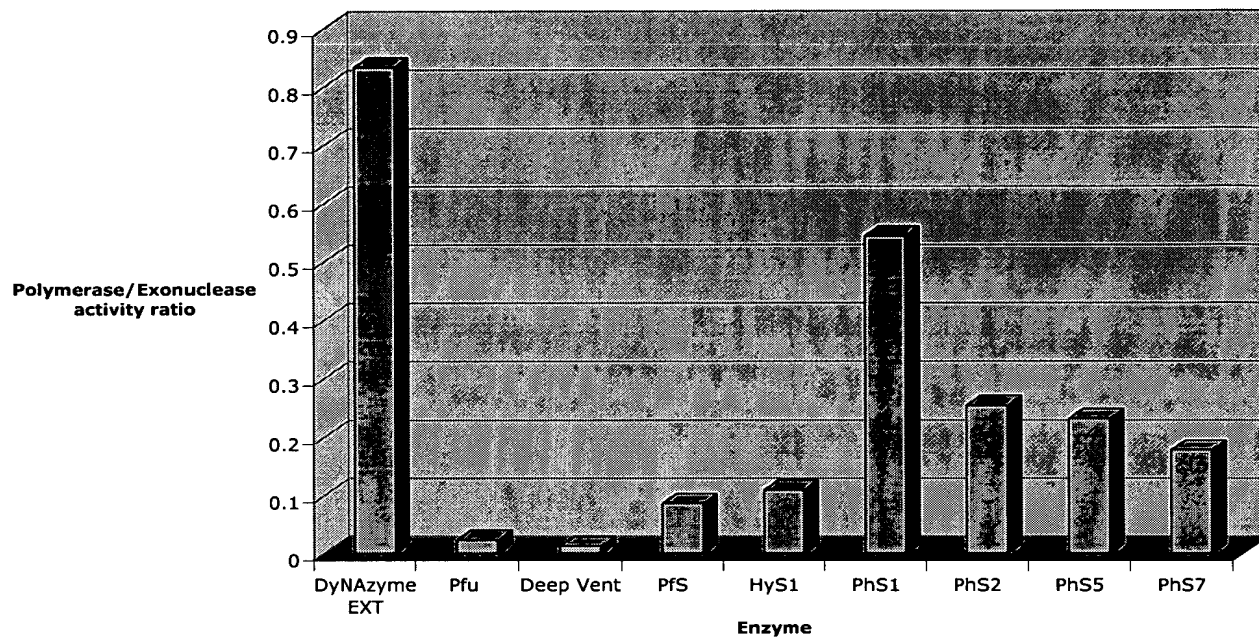




FIGURE 10

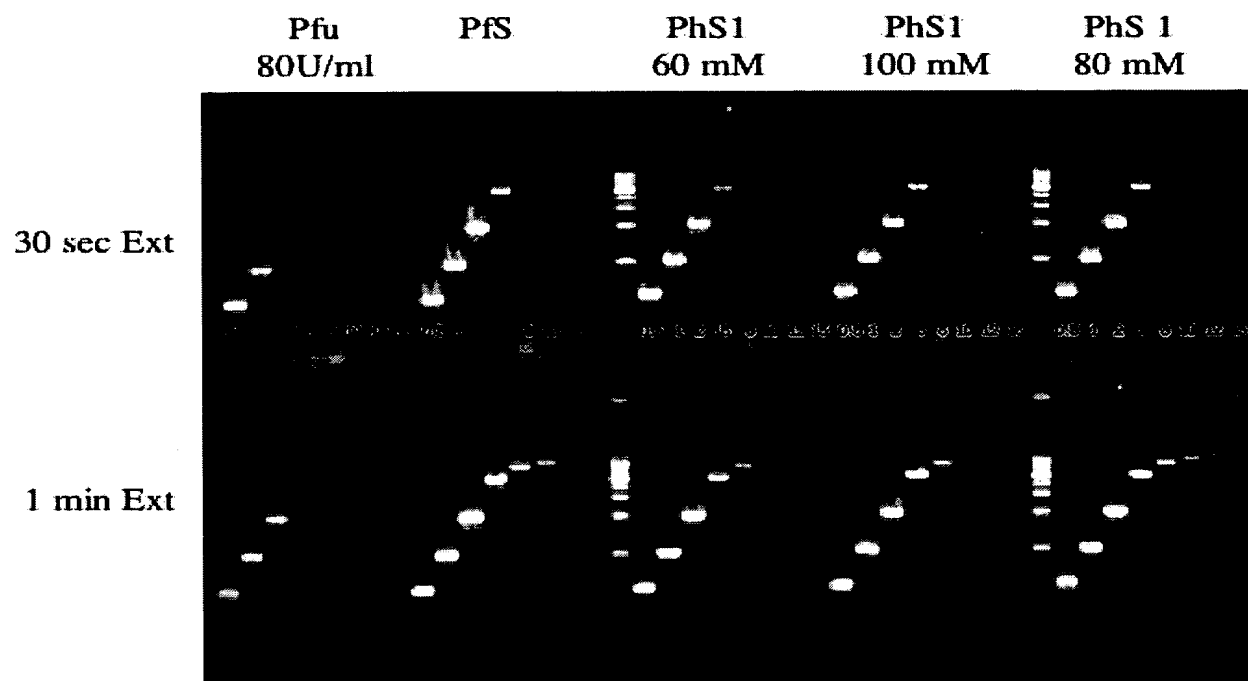
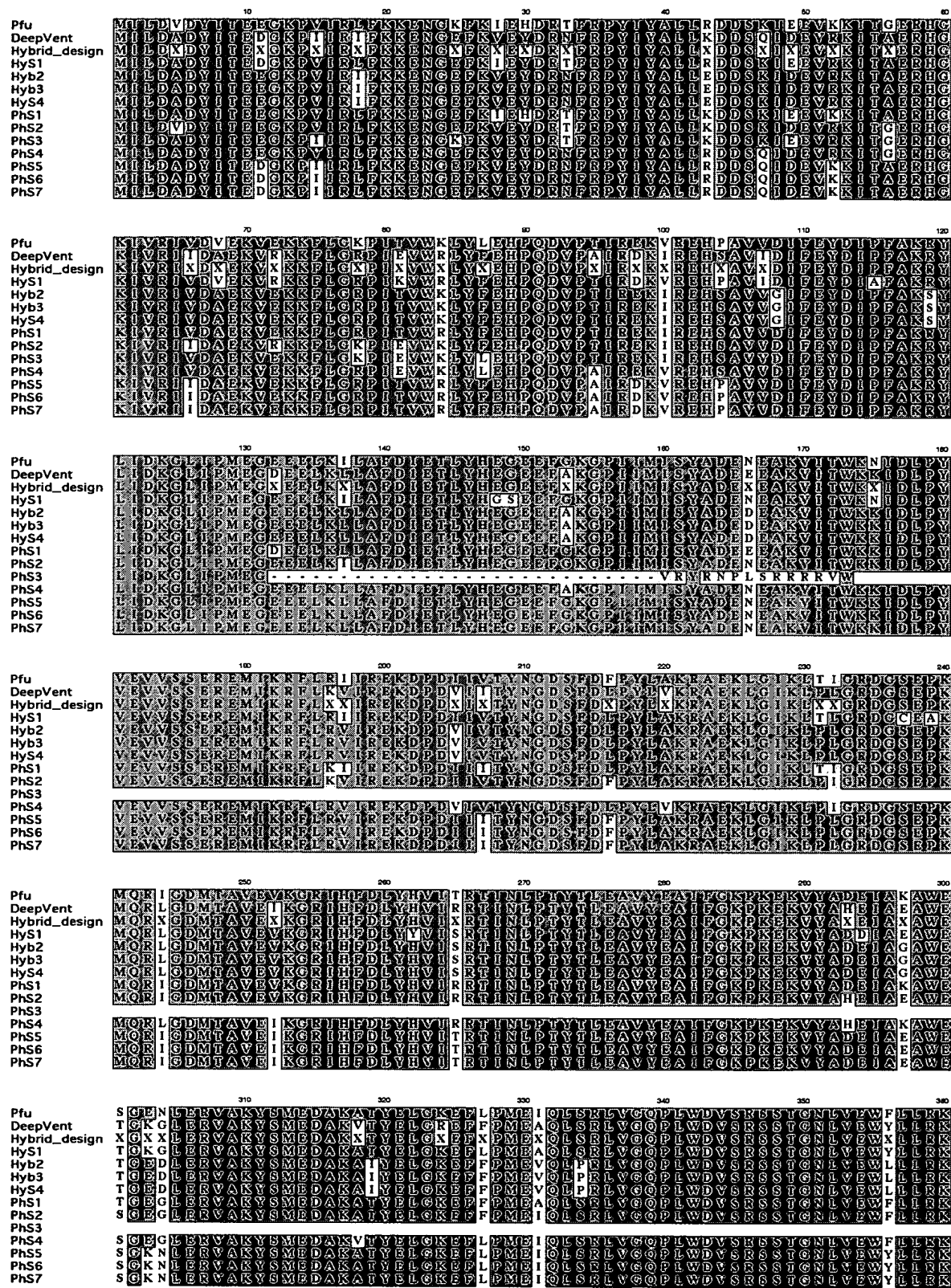


Figure 11



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Pfu  
DeepVent  
Hybrid\_design  
HybS1  
Hyb2  
Hyb3  
HyS4  
PhS1  
PhS2  
PhS3  
PhS4  
PhS5  
PhS6  
PhS7

Fdup  
 DeepVent  
 Hybrid\_design  
 HyS1  
 Hyb2  
 Hyb3  
 HyS4  
 PhS1  
 PhS2  
 PhS3  
 PhS4  
 PhS5  
 PhS6  
 PhS7

Pfu  
 DeepVent  
 Hyd1\_design  
 HyS1  
 Hyb2  
 Hyb3  
 HyS4  
 PhS1  
 PhS2  
 PhS3  
 PhS4  
 PhS5  
 PhS6  
 PhS7

505 516 529 535 540

[illegible][illegible][illegible]

	730	740	750	760	770	780
Pfu	Y D P K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
DeepVent	F D L R K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
Hybrid_design	X D X K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
HyS1	F D L R K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
Hyb2	F D L R K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
Hyb3	F D L R K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
HyS4	F D L R K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
PhS1	Y D P K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
PhS2	Y D L K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
PhS3						
PhS4	Y D P K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
PhS5	Y D L K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					
PhS6						
PhS7	Y D L K K H K Y D A E Y Y I E N Q V L P A V L R I L E A G F C Y R K E D L R W O K T K Q V G L T S W L N I K K S G T H N C					

	790	800	810	820	830	840
Pfu						
DeepVent						
Hybrid_design	N H D					
HyS1	G A T V K F K Y K G E E K E V D I S K I K K V W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
Hyb2						
Hyb3						
HyS4	G A T V K F K Y K G E E K E V D I S K I K K V W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS1	G A T V K F K Y K G E E K E V D I S K I K K V W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS2	G A T V K F K Y K G E E K E V D I S K I K K V W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS3						
PhS4	G A T V K F K Y K G E E K E V D I S K I K K V W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS5	G A T V K F K Y K G E E K E V D I S K I K K V W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS6						
PhS7	G A T V K F K Y K G E E K E V D I S K I K K V W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					

	850	860	870	880	890	900
Pfu						
DeepVent						
Hybrid_design	K Q K K N					
HyS1						
Hyb2						
Hyb3						
HyS4	K Q K K N					
PhS1	K Q K K N					
PhS2	K Q K K N					
PhS3						
PhS4	K Q K K N					
PhS5	K Q K K N					
PhS6						
PhS7	K Q K K					